

## CARBON-13 CPMAS NMR AND FTIR SPECTROSCOPIC STUDIES OF HUMIC ACIDS

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Carbon-13 NMR spectra, acquired with cross-polarization and magic angle sample spinning (CPMAS), and Fourier-transform infrared (FTIR) spectra were obtained on solid humic acids (HAs) extracted from material sampled at four selected stages in a large municipal waste treatment process. The complexity of the spectra manifests the compositional diversity of these substances. <sup>13</sup>C-<sup>1</sup>H solid-state NMR interrupted-decoupling spectroscopy aided in the examination of aromatic groups and of organic functional groups with no C-H bonds, which are typically only poorly resolved in normal <sup>13</sup>C solid state NMR spectra of such systems. Data measured with this pulse sequence also indicate that certain moieties, such as methyl groups, are quite mobile even in the solid state. NMR and FTIR spectra combined with quantitative elemental analyses allow a comparison of both differences and similarities among the HAs considered here.

Humic materials are known to be involved in a number of interrelated, chemical processes in soil and to influence plant growth directly as well as indirectly. Indirectly, humic acids (HAs) may improve soil fertility by increasing the availability of plant nutrients or by sequestering toxic, heavy metals in the soil environment. Some reports also suggest that plant roots can take up these naturally occurring heteropolymers, thereby directly affecting plant growth and metabolism (Kononova 1966).

Humic materials are receiving ever-increasing attention because of their importance in soil fertility, and because of their presence in soil conditioners and organic fertilizers. As more agricultural lands throughout the United States are treated with composted sewage sludges, a knowledge of the nature of the organic components (especially the HAs) present in such sys-

tems, as well as their modification during municipal treatment processes, has become essential.

In this contribution we focus on the humic materials obtained upon extraction of sewage samples taken at various processing stages of a large-scale metropolitan waste treatment facility. The HAs were examined by solid-state <sup>13</sup>C cross-polarization magic angle spinning (<sup>13</sup>C-CPMAS) NMR spectroscopy. Several recent reviews give additional details of this technique (Schaefer et al. 1979; Yannoni 1982; Pines et al. 1973). Fourier-transform infrared (FTIR) spectra of the HAs provide complementary data to that seen in the <sup>13</sup>C-CPMAS NMR spectra.

### EXPERIMENTAL PROCEDURE

#### *Humic acids*

The humic acids examined in this study were extracted from samples obtained at four selected stages of the sludge treatment and composting process of the Philadelphia Water Department Southwest Sludge Processing Facility. That treatment system is based upon a procedure developed by the U.S. Department of Agriculture known as the *Beltsville method* (Epstein et al. 1976). Pfeffer et al. (1984) have described the treatments and processing stages in more detail. Their report also gives the analytical characteristics of the whole samples from which the four HAs reported here were obtained. The humic acids were extracted according to literature methods (Hatcher et al. 1980) from freeze-dried samples (Fig. 1) and will be designated HA1, HA2, HA3, and HA4. All extractions were performed under N<sub>2</sub> to prevent oxidation of the HAs during the extraction procedures. The following brief descriptions of the four sludges from which the HAs were derived reflects the overall chronology of the waste treatment process

HA1: dried solid from untreated waste water  
HA2: anaerobically digested, dewatered solid

HA3: 21-d-old, dewatered, composted solid combined with two parts of wood chips on a volume basis

HA4: 3-mo-old, cured compost from which

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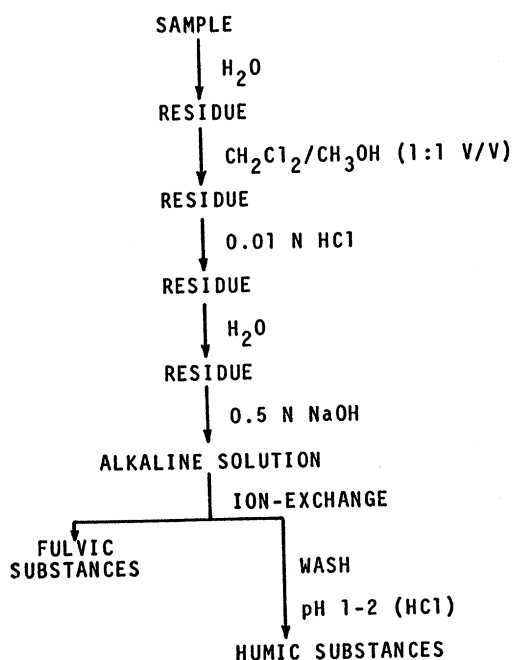


FIG. 1. Humic substance extraction scheme (all extractions were performed in an  $N_2$  atmosphere).

the wood chips have been removed by passage through compost screens

### Analyses

C, H, N, O, and methoxyl analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.<sup>2</sup>

### <sup>13</sup>C-CPMAS NMR spectroscopy

All the NMR spectra were obtained with a JEOL FX-60QS NMR spectrometer operating at a <sup>13</sup>C frequency of 15 MHz. The <sup>1</sup>H decoupling r.f. irradiation field strength was 11 G. A spectral width of 8000 Hz and a sampling of 2 k data points zero-filled to 8 k was used throughout. Chemical shifts were assigned relative to tetramethylsilane in a sealed capillary using hexamethylbenzene as a secondary reference with a shift position of 17.36 ppm for its methyl peak. Each spectrum required 20 000 to 50 000 transients. A single, matched cross-polarization contact time (Pines et al. 1973) of 0.5 ms was employed for optimal response, as determined previously for analogous systems (Pfeffer et al.

<sup>2</sup> Reference to brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

1984). Samples were spun at approximately 2.1 kHz at the magic angle of 54.7°. No spinning sidebands were detected. Interrupted <sup>13</sup>C-<sup>1</sup>H decoupling experiments, designed to enhance the <sup>13</sup>C resonances associated with nonprotonated carbon nuclei and those carbons weakly coupled to protons, were also performed (Opella and Frey 1979). A 40-μs delay without proton decoupling was inserted into the pulse sequence prior to data acquisition.

### Fourier-transform infrared spectra

All infrared spectra were measured from 4000 to 400  $cm^{-1}$  on a Nicolet 7199 Fourier-transform infrared spectrometer at ca. 2  $cm^{-1}$  resolution. The spectrometer was equipped with a Globar source, a Ge/KBr beam splitter, and a liquid-nitrogen-cooled Hg/Cd/Te detector. To ensure high signal-to-noise ratios, the spectrum of each sample was calculated from an interferogram consisting of signal-averaged data from at least 400 coadded, repetitive scans. (Total time for data acquisition was about 8 min.) Prior to Fourier transformation, each interferogram was phase-corrected, zero-filled once, and then multiplied by the Happ-Genzel apodization function. Spectra of the four humic acids were obtained from samples prepared as 7-mm-diameter KBr pellets with approximately a 1:200 weight ratio of sample to KBr. Pellets were prepared using a Harshaw Model 15 Quick Press and die set. (Use of Harshaw Stabilized KBr yielded nearly water-free pellets, as shown by spectra of blank pellets prepared under conditions analogous to those used to make the pellets of the HA samples.)

FTIR spectroscopy offers several advantages over older dispersive techniques (Bertie 1985; Griffiths 1983; Koenig 1984). Among these are enhanced signal-to-noise ratios and higher frequency accuracy and reproducibility. These advantages are particularly important when one wishes to search for relatively small changes in a complex system, as is true here.

## RESULTS AND DISCUSSION

### <sup>13</sup>C-CPMAS NMR spectroscopic analysis

Figure 2 depicts the <sup>13</sup>C-CPMAS spectra of the HAs employed in this study. HAs typically give spectra of low resolution. However, the <sup>13</sup>C spectra can be assigned to the following dominant carbon types: 0 to 50 ppm, aliphatic; 50 to 110 ppm, N- and O-substituted alkyl carbon

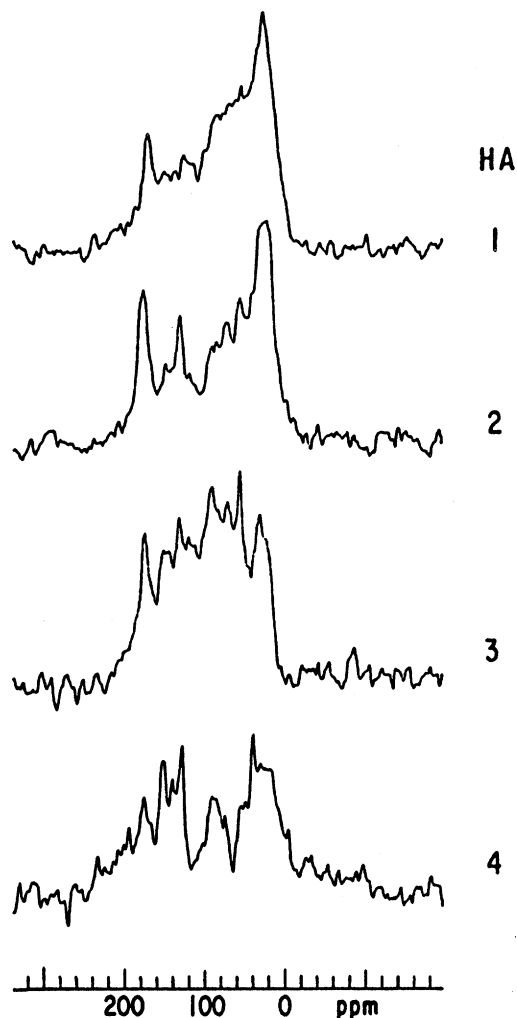


FIG. 2.  $^{13}\text{C}$ -CPMAS spectra of humic substances.

groups, including carbohydrate, ether, amine, amide, alcohol, acetal, ketal, and methoxyl functional groups; 110 to 160 ppm, aromatic and olefinic carbons; 160 to 190 ppm, carboxyl, amide carbonyl, and ester carbonyl groups; 190 to 220 ppm, ketones and aldehydes.

We note from all the spectra that the HAs studied here give rise to a dominant signal at approximately 30 ppm. This envelope of resonances corresponds to unresolved methyne, methylene, and methyl group carbon nuclei. This peak may be attributed, in part, to algal and microbial components that comprise these humic substances (Hatcher et al. 1981a). The spectral region centered around 54 ppm indicates the presence of methoxyl groups. These

functional groups are probably derived from ligninlike structures, as would be expected of HAs from terrestrial (as opposed to aquatic) sources, as well as the processes from which these latter materials were obtained. Finally, all the HAs exhibit aromatic character as revealed by the spectral features observed at approximately 135 and 155 ppm.

The relative degree of aromaticity among the HAs appears to vary as a function of the treatment process. HA3 appears to be dominated by carbohydrate carbons with resonances at approximately 74 ppm. Because wood chips have been added to the sludge at stage 3 in the treatment process, the added cellulose (as well as hemicellulose and other polymers) comprising HA3, now yields increased  $^{13}\text{C}$  signal in the carbohydrate region of the spectrum. Furthermore, upon removing residual wood chips in the final stage (Fig. 2, HA4), we observed a loss of carbohydrate intensity with a simultaneous increase in the relative aromaticity of the humic material. Analytical data, as seen in Table 1, further corroborate these trends. The H/C atomic ratio decreases by approximately 25% when comparing HAs 1 and 2 to HAs 3 and 4. The overall percentage of methoxy groups increases when the same comparison is made. Thus, increased unsaturation, aromaticity, or both as a function of processing is evident. These data also point to the fact that oxidation occurs as a function of both time and treatment, playing a key role in determining the final composition of the HAs present in sludges and composites. Carboxyl carbons are also seen in all the spectra (ca. 180 ppm). The carboxyl content decreases as the aromaticity increases. This trend is also consistent both with the NMR results discussed above and with the analytical data.

#### *Interrupted $^{13}\text{C}$ - $^1\text{H}$ decoupled spectra*

Protons exert a dipolar interaction on directly bonded carbon nuclei, as well as upon all other carbon nuclei in the immediate vicinity. The strength of this dipolar interaction varies as the reciprocal of the third power of the distance separating the proton from the carbon nucleus. In solutions such effects are averaged out to their isotropic value of zero due to rapid, random tumbling, which molecules can undergo. In the solid state, however, motion is much more restricted, and, as such, proton dipolar relaxation may result in broad  $^{13}\text{C}$  lines, especially for a

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powdered or an amorphous sample. The width of such lines may be larger than the entire frequency range of the spectrum. This problem in  $^{13}\text{C}$ -CPMAS spectroscopy is readily overcome by high-power proton-decoupling and spin-locking (Schaefer and Stejskal 1979). However, this same  $^{13}\text{C}$ - $^1\text{H}$  dipolar broadening interaction may be effectively utilized to enhance the spectral resolution of  $^{13}\text{C}$  resonances associated with nonprotonated carbon nuclei and those carbons remotely affected by protons (Opella and Frey 1979). As discussed in the literature (Opella and Frey 1979; Opella et al. 1979; Alemany et al. 1983; Pfeffer et al. 1984; Gerasimowicz et al. 1984), this technique of proton dipolar dephasing gives rise to spectra consisting only of the resonances from nonprotonated carbon nuclei and methyl groups. Some carbon-containing organic functional groups (such as methyl groups) undergo rapid, rotational motion even in the solid state. Such motion attenuates the  $^{13}\text{C}$ - $^1\text{H}$  dipolar relaxation mechanism, with the result that resonances from such carbon nuclei are attenuated, but not totally eliminated from the spectrum due to angular averaging. Nevertheless, because of their distinct chemical shifts, resonance peaks from such groups are readily assigned in  $^{13}\text{C}$ -CPMAS spectra. (See, for example, the 22-ppm resonance peak in Fig. 3.)

To facilitate comparison, Fig. 3 depicts the  $^{13}\text{C}$ -CPMAS spectra taken with interrupted  $^{13}\text{C}$ - $^1\text{H}$  decoupling of both the whole sludge samples (Pfeffer et al. 1984) and the humic acids extracted from these materials (present study). One important consideration concerning the spectra shown in Fig. 3A is that these data were obtained with no chemical modification whatsoever of the original, whole sample. The interrupted-decoupling technique permits some resolution enhancement of the substituted aromatic and remotely protonated carbon nuclei of the HA components. (See specifically the spec-

tral frequency range from ca. 135 to 155 ppm.) One can now more readily discern peaks centered around 153 ppm, which are O- or N-substituted aromatic C nuclei, as well as resonances at approximately 136 ppm, which may be assigned to aromatic or olefinic nuclei that are not electronegatively substituted (Fig. 3). The aliphatic, or paraffinic, region centered around 22 ppm is also more clearly resolved in all cases shown in Fig. 3 than in any of the standard  $^{13}\text{C}$ -CPMAS spectra of Fig. 2. The envelope of resonance peaks centered around 22 ppm may also be indicative of carbon-to-carbon branch points, rather than straight chain methylene groups.

Several other distinctions between Figs. 3A and 3B should be mentioned. Relatively higher methoxy content is seen in the later stages of the whole samples than in the corresponding HAs. In these later stages wood chips have been added, and thus the lignin contribution is somewhat greater than that observed in the humic extracts. Furthermore, all the HAs have a prominent peak at approximately 93 ppm, which is not evident in the spectra of the whole samples. The appearance of this peak in the NMR spectra in Fig. 3 is very interesting in that this resonance is clearly not seen in the corresponding spectra of the sludges from which the HAs were derived. The interrupted-decoupling technique may be enhancing  $^{13}\text{C}$  signals from relatively minor components that are present in small concentrations in the HAs. A more probable explanation, however, is that during the extraction of the sludges some chemical alteration of the samples is occurring. Such adulteration indicates that renewed emphasis must be placed on noninvasive analytical study of organic components in sludges, soils, composts, and other naturally occurring polymeric materials (Gerasimowicz et al. 1983; Gessa et al. 1983). These suppositions are currently under further investigation. Nonetheless, the interrupted-decoupling technique

TABLE 1  
Analytical characteristics of the humic samples

	Weight percentages					Atomic ratios		
	% C	% H	% N	% O	% Methoxy	H/C	C/N	C/O
HA1	38.69	6.11	5.86	25.98	2.74	1.90	7.70	1.99
HA2	41.90	6.59	6.30	24.25	2.48	1.89	7.76	2.30
HA3	46.94	5.84	6.39	29.14	5.93	1.49	8.57	2.15
HA4	46.53	5.84	4.80	28.19	3.56	1.51	11.31	2.20

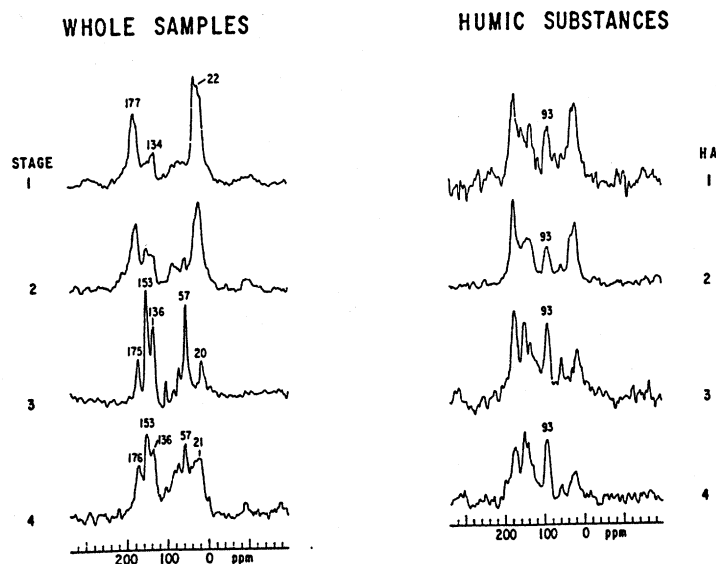


FIG. 3.  $^{13}\text{C}$ -CPMAS spectra of whole sludges and humic substances with  $^1\text{H}$ - $^{13}\text{C}$  interrupted decoupling.

does reveal the complexity of the underlying organic constituency comprising HAs. In addition, this spectroscopic tool permits a qualitative assessment of the relative degree of aromaticity present in these complex heteropolymeric matrices.

#### FTIR spectroscopic analysis

As in our previous study of sludges and sludge extracts (Gerasimowicz et al. 1983), FTIR spectroscopy serves as another qualitative tool to characterize nondestructively the principal classes of chemical groups that compose the HAs under study and complements the information obtained from solid-state  $^{13}\text{C}$ -CPMAS NMR spectroscopy.

Comparison of the FTIR spectra of HAs 1, 2, 3, and 4 (Fig. 4), shows that all exhibit a similar series of bands, indicating that the major types of chemical groups present in each of these samples are similar. The frequency positions of the bands remain remarkably constant for all four HAs (Table 1). Indeed, the frequency deviation of a given band in any one of the four spectra from the average frequency for that band reported in the table is generally less than  $3\text{ cm}^{-1}$ . Because the relative peak intensities (absorbances) of certain categories of absorption bands differ in the spectra of the four HAs (Table 1), we can obtain rough estimates of the extent to which the relative amounts of different chemical moieties vary during the waste treat-

ment process. In this section we highlight the most obvious distinctions in the four spectra and relate them to the information obtained by NMR spectroscopy.

As has been pointed out in earlier work on humic acids and related substances (Gerasimowicz et al. 1983; Boyd et al. 1979, 1980; Stevenson and Goh 1971; Theng and Posner 1967), a few groups constitute the major portion of these complex mixtures and thus produce relatively strong absorptions in the IR spectra. These groups include alkyl moieties, both methyl ( $-\text{CH}_3$ ) and methylene ( $-\text{CH}_2-$ ); amides ( $-\text{NH}-(\text{C}=\text{O})-$ ); alkoxy ( $-\text{C}-\text{O}-$ ); and hydroxyl ( $-\text{OH}$ ) (Table 2 and Fig. 4). The spectra indicate that two other important constituent groups, carbonyls ( $-\text{C}=\text{O}$ ) and aromatic rings (Ar), are also present, but, based on their relatively lower intensities, they probably occur in somewhat smaller quantities. For example, a single carbonyl group generally absorbs much more strongly than does one alkyl moiety (Bellamy 1975; Colthup et al. 1975; Simons 1978). Overall, the spectra of the four HAs from Philadelphia sludge all most closely resemble spectra of soil HAs classified by Stevenson and Goh (1971) as Type III. Samples of this type are distinguished by the presence of moderately strong absorptions near  $1650$  and  $1540\text{ cm}^{-1}$  (amide I and II) and near  $1050\text{ cm}^{-1}$  ( $-\text{C}-\text{O}-$  stretching vibrations) and by rather prominent aliphatic  $-\text{CH}$  stretching bands around ( $2800$

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to  $3000\text{ cm}^{-1}$ ) (Table 1). These easily distinguished infrared features corroborate the NMR evidence discussed in the previous section.

The general appearance of the type III HA spectra contrasts noticeably with the infrared spectra of Stevenson and Goh's (1971) type I soil HAs and with the soil HAs reported by Theng and Posner (1967). Type I HAs have spectra characterized by a rather strong carbonyl band around  $1720\text{ cm}^{-1}$  and by a second moderately intense absorption near  $1600$  to  $1610\text{ cm}^{-1}$ . Both studies attribute the first band to un-ionized carboxyl groups; a definitive assignment for the  $1600\text{ cm}^{-1}$  spectral feature seems much less certain. In contrast to type III samples (and to the four sludge-derived HAs presented here), type I HAs evince little spectral evidence for the presence of significant quantities of amide (peptide) bonds and exhibit only very modest absorptions by simple aliphatic groups.

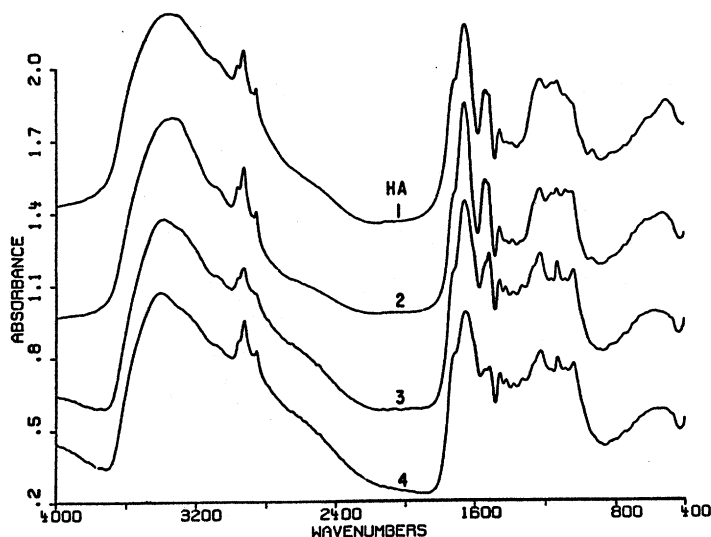
Based on the infrared spectral data, several important differences may be noted between the HAs extracted from samples taken at the four processing stages studied here. One is the relative decrease in the amide functionalities compared with the aromatic groups in the samples from the later stages of the waste treatment process. This trend is most apparent if one looks at the band assigned to the aromatic ring stretching mode near  $1516\text{ cm}^{-1}$  and compares its peak intensity to that of the neighboring absorption at about  $1540\text{ cm}^{-1}$  (Figs. 4 and 5), which is attributed to the amide II vibration of

proteins and polypeptides. For the HAs from stages 1 and 2, the  $1540\text{ cm}^{-1}$  band is stronger than the  $1516\text{ cm}^{-1}$  band; for stages 3 and 4, the relative intensities are reversed. This apparent increase in unsaturation parallels a decrease in the analytical H/C atomic ratios for HA3 and HA4 relative to the values for HA1 and HA2 (Table 1), as well as a relative increase in the amount of aromatic carbon noted in the NMR spectra. Nevertheless, the relative quantity of alkyl groups, as indicated by comparing the peak height of the  $2926\text{ cm}^{-1}$  band ( $\text{CH}_3$  sym. str.) with that of the amide I band (near  $1650\text{ cm}^{-1}$ ), decreases on going from stage 1 to stage 2; for HA3 these peaks are again in nearly the same proportion as in HA1; finally, at the last stage, there is a relative increase in the alkyl groups.

Bands at  $1263$ ,  $1222$ ,  $1125$ , and  $1034\text{ cm}^{-1}$  intensify in the later stages of processing relative to adjacent features at  $1156$  and  $1080\text{ cm}^{-1}$ . The former bands probably indicate aromatic ether linkages and/or aromatic substances with substituents in the *para*- or *ortho*- positions (Table 2). The feature at  $1080\text{ cm}^{-1}$  is strongest for HAs from the first two processing stages and is possibly associated with hydrated polyhydroxyl species, including carbohydrates.

The moderately strong shoulder near  $1720\text{ cm}^{-1}$  on the very strong amide band comes in the region expected for ketones and carboxylic acid dimers. A very weak shoulder around  $925\text{ cm}^{-1}$  gives further evidence of the latter species.

FIG. 4. FTIR spectra of humic substances ( $4000$  to  $400\text{ cm}^{-1}$ ).



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TABLE 2

Observed infrared bands of humic acids from Philadelphia sludge sampled at four different stages during the processing treatment

Band, <sup>a</sup> cm <sup>-1</sup>	Proposed assignment <sup>b</sup>	Relative intensity <sup>c</sup>			
		HA1	HA2	HA3	HA4
3400br	—OH stretch (hydrogen-bonded hydroxyl)	vs	vs	vs	vs
3290sh	amide A (hydrogen-bonded —NH stretch)	ms	s	ms	ms
3070	—CH— stretch (aromatic; olefinic)	vw	vw	vw	vw
2958	—CH <sub>3</sub> asym stretch	w	mw	w	w
2926	—CH <sub>2</sub> — asym stretch	mw	m	mw	mw
2873	—CH <sub>3</sub> sym stretch	vw	vw	vw	vw
2855	—CH <sub>2</sub> — sym stretch	w	mw	w	w
1718sh	—C=O stretch (ketones, acid dimers)	ms	m	ms	ms
1653	amide I (hydrogen-bonded —C=O stretch)	vs	vs	vs	vs
1630sh	—C=C— stretch ( <i>cis</i> -alkene) ?				
1595sh	aromatic ring stretch				
1539	amide II (hydrogen-bonded —NH bend)	ms	ms	ms	ms
1517	aromatic ring stretch ( <i>para</i> - and <i>ortho</i> -disubstituted)	ms	ms	s	ms
1465sh	—CH <sub>2</sub> — bend;				
1455	—CH <sub>3</sub> asym bend; —CH <sub>3</sub> sym bend (methoxy); aromatic ring stretch ( <i>ortho</i> -disubstituted)	m	m	m	ms
1420	aromatic ring stretch ( <i>ortho</i> -disubstituted)	m	m	m	ms
1384	—CH <sub>3</sub> sym stretch, at 2 or 3° carbon	m	m	m	ms
1377	—CH <sub>3</sub> sym stretch, at 1° carbon	m	m	m	ms
1330	— ?	m	m	m	ms
1265sh	—C—O— stretch (aromatic ether, ArOR; acid dimers with electron-withdrawing groups); amide III (—C—N— stretch)	ms	m	ms	ms
1223	— ?	s	ms	s	s
1156	— ?	s	ms	m	m
1125	aromatic ring bending ( <i>para</i> - and <i>ortho</i> -disubstituted); C—O—C stretch (alkyl ethers)	s	ms	ms	ms
1080	—C—O— stretch (hydrated polyols and carbohydrates)	ms	ms	m	m
1034	—C—O— stretch (aromatic ether, ArOR)	m	ms	ms	ms
925sh	—OH bend (carboxylic acid dimer)	vw	vw	vw	vw?
825	CH bend (aromatic, <i>para</i> -disubstituted)	w	w	vw	vw
805		w	w	vw	vw
742		w	w	vw	vw
699	— ?	w	w	w	w
620br	— ?	mw	mw	mw	mw
590	— ?	—	—	mw	mw
540	— ?	—	—	—	w
520br	ring bend (aromatic, <i>para</i> -disubstituted) ?	m	m	mw	mw
470br		mw	mw	mw	mw

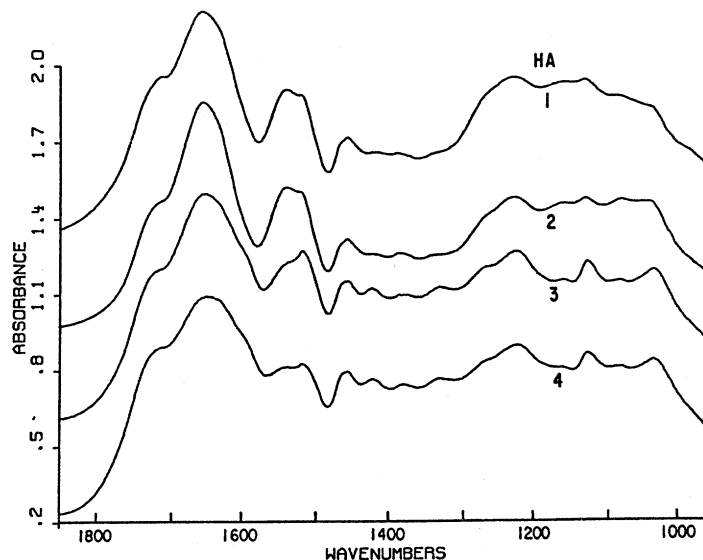
<sup>a</sup> The frequencies reported here are average values for the observed bands of each of the four spectra. Except for broad (br) or overlapping bands (shoulders, sh) these values are probably accurate to  $\pm 1$  cm<sup>-1</sup>. In general the deviation of the frequency of any individual band from the average values reported here is less than 3 cm<sup>-1</sup>.

<sup>b</sup> Bellamy 1975; Colthup et al. 1975; Simons 1978.

<sup>c</sup> Relative peak intensity: vs, very strong; s, strong; ms, medium strong; m, medium; mw, medium weak; w, weak; vw, very weak; —, not observed.

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FIG. 5. FTIR spectra of humic substances (1850 to 950  $\text{cm}^{-1}$ , expanded scale).



(Because the HAs were acid-ion exchanged and then lyophilized from solutions at pH 2, carboxyl groups when present will generally remain protonated.)

### CONCLUSIONS

On the basis of the foregoing discussion and data, we conclude that  $^{13}\text{C}$ -CPMAS NMR and FTIR spectroscopies indeed complement one another in terms of ascertaining and corroborating the qualitative structural and functional characteristics of HAs. Both spectroscopic techniques confirm that the distribution of organic functional moieties within these samples corresponds to that observed for HAs of terrestrial origin, as opposed to the distribution of organic groups seen in HAs from aquatic or marine sources (Hatcher et al. 1981a, b). These two spectroscopic methods unquestionably indicate that the HAs derived from samples taken at specific points in the municipal waste treatment process studied here clearly differ from one another with respect to the relative quantities of any given organic functional group observed. Yet, there is no marked distinction among the four HAs studied with respect to the identity of the major categories of functional groups present in the samples. That is, the waste treatment process does not, in the main, drastically alter the overall nature of these heterogeneous materials. Nevertheless, the spectra demonstrate that these four HAs studied are unmistakably different from many of the HAs isolated from

various types of soils (Stevenson and Goh 1971; Theng and Posner 1967).

In many instances,  $^{13}\text{C}$ -CPMAS NMR spectroscopic data lend themselves somewhat more to quantitative analysis than do the FTIR results. In particular, the relative aromatic nature of the HAs was more evident from the NMR spectra and could not be seen as readily from the FTIR data. Furthermore, the possibility that chemical modification of the samples occurred during the extraction of the HAs was more apparent in the NMR spectra obtained with  $^1\text{H}$ - $^{13}\text{C}$  interrupted-decoupling. FTIR spectroscopy, however, proved useful in differentiating certain features of the HAs that were less feasible to assess by NMR techniques alone. For example, although carbonyl resonances are seen in the  $^{13}\text{C}$  NMR spectra, some are not well resolved due to the large chemical shift anisotropy of this functionality. FTIR was useful in differentiating and assigning the carbonyl bands, due to carboxyl and amide groups, that were broadened in the NMR spectra. The combination of quantitative elemental analysis supplemented by modern, nondestructive NMR and FTIR characterization has been demonstrated to have potentially wide biochemical implications in the study of the nature and properties of humic substances and other complex heterogeneous matrices.

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